Design, Synthesis, and Biological Evaluation of Matrix Metalloproteinase Inhibitors Derived from a Modified Proline Scaffold

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The synthesis and structure−activity relationship (SAR) studies of a series of proline-based matrix metalloproteinase inhibitors are described. The data reveal a remarkable potency enhancement in those compounds that contain an sp^2 center at the C-4 carbon of the ring relative to similar, saturated compounds. This effect was noted in compounds that contained a functionalized oxime moiety or an exomethylene at C-4, and the potencies were typically <10 nM for MMP-3 and <100 nM for MMP-1. Comparisons were then made against compounds with similar functionality where the C-4 carbon was reduced to sp^3 hybridization and the effect was typically an order of magnitude loss in potency. A comparison of compounds **14** and **34** exemplifies this observation. An X-ray structure was obtained for a stromelysin-inhibitor complex which provided insights into the SAR and selectivity trends observed within the series. In vitro intestinal permeability data for many compounds was also accumulated.

Introduction

An important subset of proteinase enzymes consists of the matrix metalloproteinases (MMPs) which are characterized by the presence of a zinc ion in the active site. This family of enzymes is composed of 17 known members, and new members have been identified with startling regularity in recent years. These endo proteinases are involved primarily in tissue remodeling and include the collagenases, gelatinases, and stromelysins. Under physiological conditions, the proteolytic activity of the MMPs provides a means for the degradation of the extracellular matrix tissue to allow for normal remodeling events such as tissue turnover, wound healing,[1] and angiogenesis.[2] This activity is regulated by the tissue inhibitors of matrix metalloproteases (TIMPS). Several pathological conditions (osteoarthritis,[3] rheumatoid arthritis,[4] periodontal disease,[5] multiple sclerosis,[6] tumor metastasis[7]) have been linked to overexpression of the MMPs. The inhibition of the MMP enzymes thus represents an attractive target for medicinal intervention in degenerative conditions where excessive tissue remodeling plays a key role. This opportunity has stimulated extensive research in both academic and industrial laboratories toward the development of small molecule MMP inhibitors, and efforts along these lines have been extensively reviewed.[8]

Our group and others have been developing MMP inhibitors for a number of years, and numerous scaffolds have been reported in the literature including succinamides[9] (Marimastat), linear sulfonamides[10] (CGS-27023A), and heterocyclic sulfonamides[11] (AG-3340) (Figure 1). While much of our earlier work was based on succinamide type structures, our recent effort has been directed toward heterocyclic sulfonamide analogues. In the course of our studies, we explored scaffolds which could be readily prepared from commercially available materials, and we observed a striking potency boost in compounds derived from *cis*-hydroxy-D-proline where the C-4 center was oxidized to form an sp^2 center. The account below will focus primarily on the SAR and selectivity profiles for compounds which display this unexpected potency enhancement.

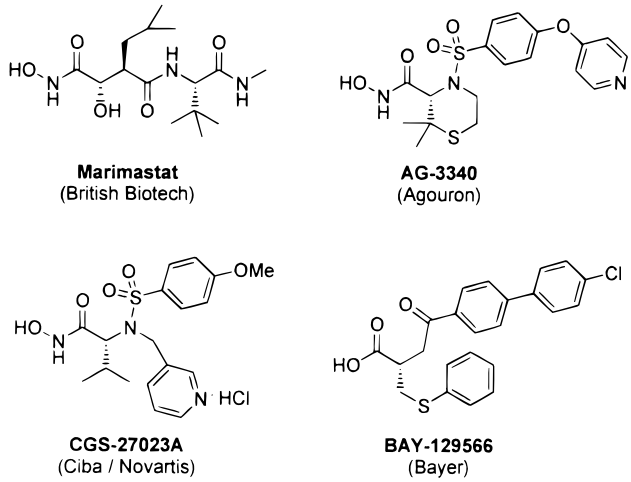

Figure 1.

Chemistry

All compounds were derived from *cis*-4-hydroxy-D-proline **1** as exemplified in Scheme 1. A routine sequence of esterification, sulfonation, and oxidation provided ketones of type **2** which served as pivotal

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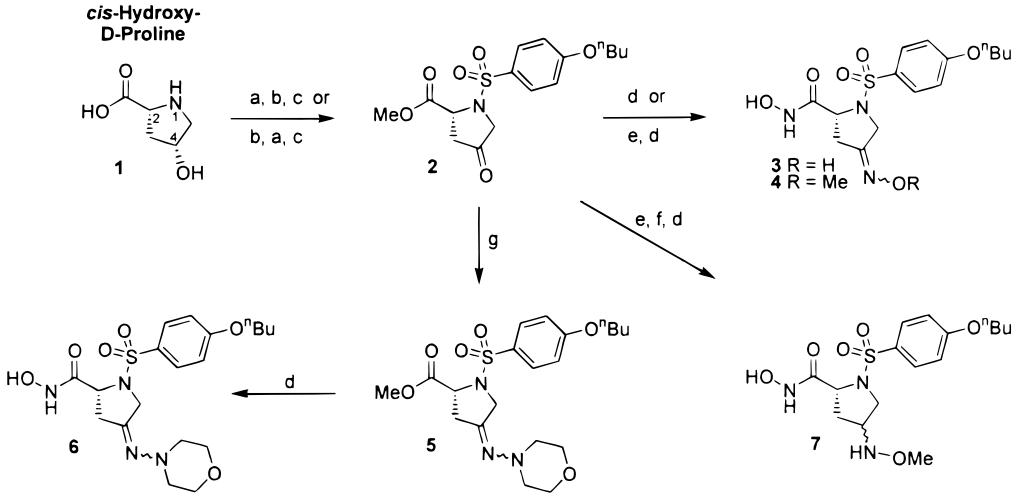
Scheme 1[a]



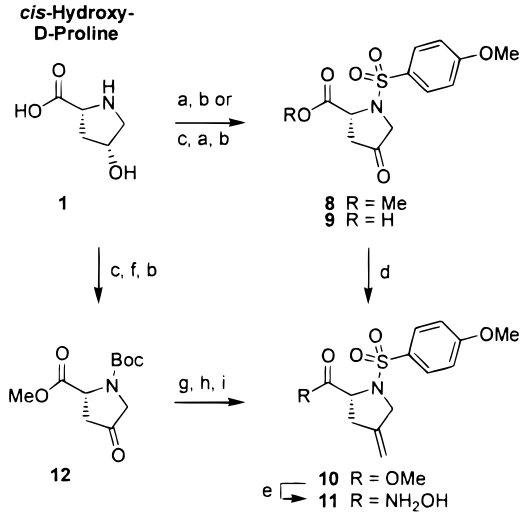
[a] Reagents and conditions: (a) MeOH, $SOCl_2$; (b) $ClSO_2C_6H_4O^nBu$, dioxane, H_2O, Et_3N; (c) Jones oxidation; (d) NH_2OH, KOH, MeOH; (e) $MeONH_2$·HCl, MeOH, dioxane, NaOAc; (f) $NaCNBH_3$, MeOH, concd HCl; (g) 1*N*-aminomorpholine, MeOH, dioxane, NaOAc.

intermediates for further elaboration. Treatment of **2** with excess basic hydroxylamine[12] gave oxime hydroxamates of type **3** directly; alternatively, sequential treatment with a controlled amount of alkoxy- or aryloxyamine followed by treatment with hydroxylamine provided functionalized oximes of type **4**. The oximes were isolated as mixtures of *E* and *Z* isomers which could not be easily separated by HPLC. Hydrazones of type **6** were similarly prepared from ketone **2**. Treatment of **2** with a disubstituted hydrazine gave hydrazone **5**. Subsequent treatment with hydroxylamine then provided the desired hydroxamate **6**. These hydrazones were isolated and purified as variable mixtures of *E*/*Z* isomers, but the compounds tended to slowly darken and decompose over time.

A number of conditions were investigated to reduce the oxime functionality and convert C-4 from an sp^2 to an sp^3 center. Both hydrogenation and hydride reduction conditions were investigated with limited success. Hydride addition with cyanoborohydride and catalytic HCl ultimately emerged as the most synthetically efficient method for the transformation. The methoxylamine **7** was prepared using this methodology and was obtained as a mixture of diastereomers (2:1, unassigned, not separated). The mixture was converted to the corresponding hydroxamic acid with basic hydroxylamine.

Direct analogues of the oxime moiety were sought which also contain an sp^2 carbon at C4, and thus various olefinic type compounds were prepared as shown in Scheme 2. The exomethylene moiety in compound **10** was introduced from keto-sulfonamide **8** using standard Wittig conditions; however, the yield was generally <25% and highly variable. This is likely due to a propensity of the sulfonamide moiety to eliminate under basic conditions since various elimination products were observed in these reactions. Similar conditions were also applied to the keto acid **9** to discourage such eliminations, but yields turned out to be even lower. Better results were achieved with the Boc-protected ketoproline ester **12**. Standard Wittig conditions were successful at delivering the target olefination, albeit in a very modest 20% yield. Much better results were achieved under equilibrating conditions via the Dauben-Conia modification of the Wittig reaction.[13] These conditions were successful in delivering the desired olefination product and worked especially well with stabilized ylides such as $PhCH_2PPh_3$ and $CNCH_2PPh_3$; however, only the exomethylene case was carried forward to properly functionalized hydroxamates of type **11** for biological testing. Other nucleophilic olefination methods were also pursued without satisfactory results.[14]

Scheme 2[a]

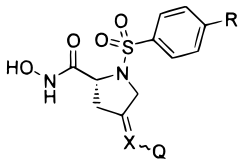


[a] Reagents and conditions: (a) $ClSO_2C_6H_4OMe$, dioxane, H_2O, Et_3N; (b) Jones oxidation; (c) MeOH, $SOCl_2$; (d) Ph_3PCH_3Br, LiHMDS, THF; (e) NH_2OH, MeOH; (f) Boc_2O, acetone, H_2O, Et_3N; (g) Ph_3PCH_3Br, THF, *tert*-amylOK, *tert*-amylOH; (h) TFA, CH_2Cl_2; (i) $ClSO_2C_6H_4OMe$, NEt_3, CH_2Cl_2.

Biological Results and Discussion

SAR Effects of the Oxime- and Hydrazone-Bearing Pyrrolidines. All compounds were assayed in vitro for the inhibition of truncated collagenase-1 (MMP-1),[15] gelatinase-A (MMP-2),[15] stromelysin (MMP-3),[16] matrilysin (MMP-7),[15] and collagenase-3 (MMP-13).[15] Several structure−activity relationships become apparent upon inspection of the binding data. Particularly noteworthy is the consistently high potency of the

Table 1. MMP Inhibition and Absorption Data for Oxime, Hydrazone, and Exomethylene Derivatives



				MMP IC_{50} (nM)[a]					
cmpd	Q	X	R	−1	−2	−3	−7	−13	% abs
13	-OH	-N	-OMe	17	0.2	5	nd	0.2	29−35
3	-OH	-N	-O^{n}Bu	119	0.2	5	307	0.3	2−5
14	-OMe	-N	-OMe	10	<1	3	82	<0.5	78−81
15	-OMe	-N	-OEt	22	0.3	16	1058	0.7	23−42
16	-OMe	-N	-O^{n}Pr	13	<0.4	2	381	<1	nt
4	-OMe	-N	-O^{n}Bu	26	<1	1	89	0.3	28−36
17	-OMe	-N	-OCH_2CH_2OMe	109	0.5	15	612	<0.4	nt
18	-OMe	-N	-OPh	3	<0.4	2	60	<0.4	15−18
19	-OMe	-N	-OC_6H_4F	3	<0.4	<1	<24	0.8	nt
20	-OMe	-N	-O-4-Pyr	81	0.3	5	922	<0.2	50−68
21	-OEt	-N	-O^{n}Bu	39	<0.4	2	39	0.5	10−17
22	-O^{t}Bu	-N	-OMe	20	0.7	9	263	<0.5	13−19
23	-O^{t}Bu	-N	-O^{n}Bu	74	0.5	11	294	0.4	nd
24	-O^{t}Bu	-N	-O-4-Pyr	26	<0.4	5	785	<0.4	10−18
25	-O^{t}Bu	-N	-OC_6H_4F	24	<0.4	<1	1032	0.2	insol.[c]
26	-O^{i}Bu	-N	-O^{n}Bu	75	<0.4	12	420	<0.1	nt
27	-OPh	-N	-O-4-Pyr	32	nd	5	785	0.4	nd
28	-OCH_2Ph	-N	-O^{n}Bu	127	<0.4	17	490	0.1	nt
29	-*N*-piperazine	-N	-O^{n}Bu	184	nd	3	343	<1	10−19
6	-*N*-morpholine	-N	-O^{n}Bu	293	nd	2	196	<1	1
11	-H	-CH	-OMe	90	nd	14	12300	4	46−66
30	-H	-CH	-O^{n}Pr	155	2	16	2546	1	nt
31	-H	-CH	-O^{n}Bu	329	0.8	15	1082	0.7	nt
32	-H	-CH	-OCH_2CH_2OMe	1548	9	69	>9994	11	nt
33	-H	-CH	-OPh	15	0.4	14	53	<0.4	nt

[a] See Experimental Section for enzyme assay details. Standard deviations were typically ±60% of the mean or less. [b] Absorption values were predicted from in vitro rat ileum permeability studies (see Experimental Section). [c] Not soluble in aqueous buffer used in transport studied. nt, not tested. nd, none detected.

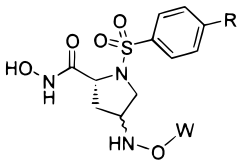
oxime- and hydrazone-bearing compounds against MMPs -2, -3, and -13 as seen in Table 1. All of these compounds were single nanomolar or picomolar inhibitors of these MMPs and demonstrated very little variation in potency as functional groups were altered. MMPs -1 and -7 showed a broader range of SAR over the series and could be manipulated somewhat to give compounds with unique enzymatic profiles. The high potency observed with this series seemed to be highly dependent on the presence of a hydroxamic acid. Replacement of the hydroxamic acid with a carboxylic acid resulted in complete loss of potency (IC_{50} for all enzymes > 10 μM).

Variation of the aromatic sulfonamide substituent (R) had moderate effects on the potency against MMP-1 and tended to give compounds with lower potency as the chain length of the substituent increased (compare **13** vs **3**, **14** vs **4**, and **22** vs **23**). This is consistent with the finding that this substituent fits into the P1′ pocket which is much more shallow for MMP-1 and -7 than the other enzymes of the family.[17] Compounds with aryl groups in this position such as **18** and **19** tended to increase the potency for all enzymes and produce broad spectrum inhibitors. Heterocyclic sulfonamides including the pyridine in **20** were less potent against MMPs -1 and -7 than the analogous aryl substituents.

The oxime hydroxyl substituents (Q) tended to exert little influence on potency within the series, and the enzymes seemed to tolerate hydroxy, alkoxy, and aryloxy groups equally well. There appeared to be a slight decrease in potency for MMP-1 with the hydrazone substituents in **29** and **6**. A modest decrease in potency for MMPs -1, -3, and -7 was observed when the oxime moiety was exchanged for an exomethylene group, indicating that the heteroatoms do play a role in binding with the enzyme. Particularly noteworthy is the incremental increase in selectivity of MMP-3 over MMP-1 observed with the exomethylene-containing compounds as the sulfonamide (R) substituent increased in chain length. The ratio of MMP-1/MMP-3 increased from 6 to 10 to 21 as the substituents were changed from methyl to *n*-propyl to *n*-butyl in compounds **11**, **30**, and **31**, respectively.

SAR Effects of a Reduced sp^2 Center on the Pyrrolidine Ring. A very interesting observation was made upon reduction of the oxime double bond to a single bond to give compounds of the type shown in Table 2. The binding potency observed with these compounds decreased by roughly 1 order of magnitude across the entire family of enzymes (compare **4** vs **7**). As with the oxime compounds, extending the chain length of the S1′ substituent (R) tended to decrease the potency of MMP-1. Modifications of the hydroxyl substituent (W) had little effect on the potency of this subset of molecules as exemplified in the comparison of **34** vs **37**. On the basis of these results, the presence of an sp^2 center at C-4 of the pyrrolidine ring is apparently responsible, at least in part, for the extremely potent nature of the oxime-bearing molecules, and this may be due to the influence of an sp^2 center on the conformation of the pyrrolidine ring.

Table 2. MMP Inhibition and Absorption Data for Substituted Hydroxylamine Derivatives



compd	W	R	MMP IC_{50} (nM)[a] −1	−2	−3	−7	−13	% abs[b]
34	-Me	-OMe	212	5	19	3393	3	12−15
7	-Me	-O^{n}Bu	790	1	21	2083	2	14−17
35	-Me	-OCH_2CH_2OMe	4235	11	222	6724	8	nt
36	-Me	-OPh	86	<0.4	6	1792	<0.4	nt
37	-tBu	-OMe	255	4	20	5235	3	nt

[a] See Experimental Section for enzyme assay details. Standard deviations were typically ±60% of the mean or less. [b] Absorption values were predicted from in vitro rat ileum permeability studies (see Experimental Section). nt, not tested.

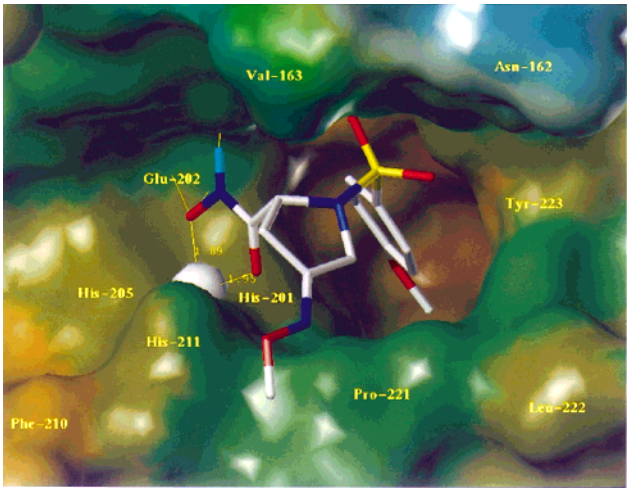


Figure 2. Structure of stromelysin-**14** complex.

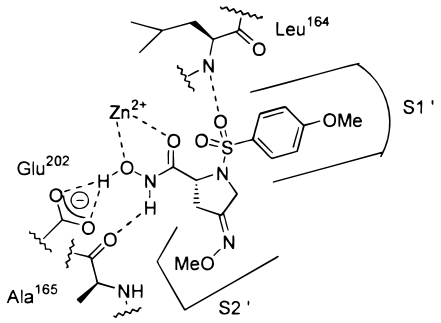


Figure 3. Schematic diagram of binding interactions between **14** and truncated stromelysin.

Structure of Stromelysin-Inhibitor Complex.[18] A crystal structure of the truncated stromelysin-**14** complex was obtained from a soaked crystal sample and solved using the molecular replacement method and is shown in Figure 2. This structure has provided insight into the key enzyme−substrate interactions that were playing a role in binding, and some of these are represented schematically in Figure 3. Many of the binding modes observed were consistent with others which have been described in the literature.[19] The hydroxamic acid plays a dominant role in the enzyme−substrate interactions, and this is due to a chorus of closely knit interactions which work in harmony to anchor the inhibitor. The ensemble is rooted by the bidentate chelation with the Zn^{2+} ion to both the carbonyl oxygen at 1.95 Å and the *N*-hydroxyl oxygen at 1.89 Å. The hydroxamate nitrogen then contributes a hydrogen bond to Ala-165 at 1.97 Å, and a final hydrogen bond from Glu-202 anion to the hydroxyl hydrogen at 2.69 Å completes the binding network.

The sulfonamide moiety also possesses favorable binding interactions with the enzyme. A dominant feature of this group is a strong hydrogen bond from the R-configured sulfonamide oxygen which is within bonding distance of both Leu-164 at 1.90 Å and Ala-165 at 2.33 Å.[20] The relative orientation of this oxygen on the sulfonamide moiety allows for proper alignment of the methoxyphenyl group which fits nicely into the deep P1′ pocket where it experiences a very compatible hydrophobic binding environment.

While the binding roles of the sulfonamide and hydroxamate groups appear to be well explained by the crystal structure in Figure 2, there seem to be no obvious interactions which can explain the potency increase that was observed with molecules possessing an sp^2 center at C-4. The oxime moiety appears to extend into the S2′ pocket on the surface of the enzyme which is a very hydrophobic environment. The flat SAR in this part of the molecule tends to suggest that this region of the molecule is contributing little to the overall binding, so we speculate that the sp^2 orientation at C-4 alters the conformation of the ring to a more efficiently bound orientation.

In Vitro Absorption. The relative peroral intestinal absorption potential (% abs.) shown in Tables 1 and 2 were predicted from in vitro rat ileum transport studies.[21] The results suggested that there was a general trend toward lower absorption when the substituent on the molecules became larger. Examples of this phenomenon can be seen in series **13** and **3**, series **20**, **24**, and **27**, and series **14**, **15**, **4**, and **18**. However, compound **20**, a pyridyl derivative, showed a greater absorption potential than would be anticipated based upon size alone (compare to **18**). A recent report has shown that several pyridyl-substituted compounds have a greater transcellular permeability than the corresponding phenyl-substituted molecule by an, as yet, undetermined mechanism.[22] This phenomenon is also supported when comparing compounds **22** and **24**. The addition of hydrophilicity in the molecules also reduced the absorption potential as exemplified by comparing compounds **29** and **6**. Two compounds, **23** and **27**, showed no

detectable transport, which may be attributed, in part, to the low solubility of these molecules. In fact, significant amounts of these two compounds partitioned into and remained in the intestinal tissue during the experiment. Moreover, there was some evidence that other compounds from this general class were substrates for the various concentration-dependent efflux systems (including P-glycoprotein), encoded by the MDR1 gene, present in the enterocytes of the intestinal tract. Further characterization would be necessary to better understand potential solubility, tissue partitioning behavior, and/or efflux mechanisms on overall peroral absorption.

Conclusion

In summary, we have described an extremely potent series of heterocyclic MMP inhibitors which depend on the presence of an sp^2 configuration at the C-4 center of the proline ring for added potency. The compounds can be prepared through a brief synthetic sequence which relies on *cis*-4-hydroxy-D-proline as a chiral template. The resulting oxime- and exomethylene-bearing inhibitors are very potent inhibitors for MMP-3 and -13 and are moderately selective against MMPs -1 and -7. Substitutions on the oxime portion of the molecule exerted little influence on potency, while substitutions on the sulfonamide portion of the molecule produced a more pronounced effect and could be manipulated somewhat to alter selectivity profiles of molecules. This is consistent with X-ray data which shows that the sulfonamide portion of the molecule goes into the P1′ pocket of the enzymes which is known to be shallower for MMPs -1 and -7 than other enzymes in the family. Studies into the relative absorption potentials of various members of the series helped to identify characteristics which serve to increase their absorption potential.

Experimental Section

General. All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. The following solvents and reagents have been abbreviated: tetrahydrofuran (THF), ethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), methanol (MeOH). All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60F-254, E. Merck) and visualized with UV light, iodine vapors, or 5% phosphomolybdic acid in 95% ethanol. Final compounds were typically purified either by flash chromatography on silica gel (E. Merck, 40−63 mm) or by preparative reverse-phase high-pressure liquid chromatography (RP-HPLC) using a Waters model 4000 Delta Prep instrument equipped with a Waters Symmetry preparative steel column (C-18, 19 m × 300 mm) as the stationary phase. The mobile phase employed 0.1% formic acid with acetonitrile as the organic modifier. Both isocratic and linear gradient methods were used as appropriate, and the flow rate was 20 mL/min. Analytical purity was assessed by RP-HPLC using a Waters 600 system equipped with a diode array spectrometer (*ì* range 200−400 nm). The stationary phase was a Waters Symmetry C-18 column (4.6 mm × 200 mm). The mobile phase employed 0.1% formic acid with acetonitrile as the organic modifier and a flow rate of 1.0 mL/min. Analytical data is reported as retention time, t_R, in minutes (% acetonitrile, time, flow rate).

^{1}H NMR spectra were recorded on a Varian Unity-300 instrument. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Low-resolution mass spectra (MS) were recorded on a Micromass Platform quadrupole mass spectrometer. Mass spectra were acquired in either the positive or negative ion mode under electrospray ionization (ESI). Combustion analyses were performed internally.

Human synovial proMMP-3 was obtained from Dr. Hideaki Nagase, University of Kansas Medical Center, Kansas City, KS. Human fibroblast proMMP-1, human MMP-9, and human recombinant MMP-7 catalytic domain were obtained from Dr. Howard Welgus, Jewish Hospital, St. Louis, MO. Human recombinant MMP-8 catalytic domain was obtained from Dr. Harald Tschesche, University Bielefeld, Bielefeld, Germany. Human recombinant proMMP-2 was purified from CHO cells as described below. Human recombinant truncated MMP-3 and truncated MMP-1 were purified from *Escherichia coli* cells as described below. Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 was purchased from Bachem Bioscience, King of Prussia, PA. Phenoxyphenylsulfonyl chloride[23] and (pyrid-4-yl)oxyphenylsulfonyl chloride[24] were prepared following literature procedures.

Methyl 1*N*-(4-*n*-Butoxyphenylsulfonyl-(4*R*)-hydroxypyrrolidine-(2*R*)-carboxylate (2a). *cis*-4-Hydroxy-D-proline (14.8 g, 112.95 mmol) was mixed with water:dioxane (1:1, 90 mL), triethylamine (39.3 mL, 282 mmol), and 4-(dimethylamino)pyridine (1.3 g, 11.3 mmol). The 4-(*n*-butoxy)phenylsulfonyl chloride (29.5 g, 118.6 mmol) was then added, and the mixture was stirred for 14 h at room temperature. The mixture was then concentrated and diluted with EtOAc and 1 N HCl. The layers were separated, and the organic layer was washed twice with 1 N HCl and once with brine, dried over $MgSO_4$, filtered, and evaporated to give 37.4 g of solid material which was dissolved in MeOH (200 mL). Thionyl chloride (20 mL, 272 mmol) was added dropwise, and the resulting mixture was stirred for 14 h. The mixture was then evaporated to dryness to give a white solid which was sufficiently pure to carry forward without purification. ^{1}H NMR ($CDCl_3$): δ 1.01 (t, J = 7.4 Hz, 3H), 1.38−1.52 (m, 2H), 1.66−1.82 (m, 2H), 2.01−2.20 (m, 2H), 2.72−2.94 (m, 1H), 3.24−3,36 (m, 1H), 3.44−3.58 (m, 1H), 3.58 (s, 3H), 3.98−4.08 (m, 2H), 4.12−4.19 (m, 2H), 6.98 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H). ESI MS: *m/z* 375 $[M + NH_4]^+$, 358.3 $[M + H]^+$.

Methyl 1*N*-(4-*n*-Butoxyphenylsulfonyl)-4-oxo-pyrrolidine-(2*R*)-carboxylate (2). An 8 N solution of Jones reagent was prepared (*Oxidations in Organic Chemistry*, p 273). The alcohol **2a** (40 g, 112 mmol) was dissolved in 300 mL of acetone and cooled to 0 °C. Jones reagent was added (120 mL, 960 mmol) (color changed from orange-red to green), and the mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with water and extracted three times with EtOAc. The organic layers were washed three times with water and once with brine, dried over magnesium sulfate, and evaporated. The product was crystallized from EtOAc to give the desired product as a solid. ^{1}H NMR ($CDCl_3$): δ 1.01 (t, J = 7.3 Hz, 3H), 1.43−1.63 (m, 2H), 1.74−1.98 (m, 2H), 2.56 (dd, J = 8.1, 3.1 Hz, 1H), 2.80 (dd, J = 18.2, 9.2 Hz, 1H), 3.68 (s, 3H), 3.78−3.81 (m, 2H), 4.02 (t, J = 6.6 Hz, 2H), 4.78 (dd, J = 9.1, 3.1 Hz, 1H), 7.01 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H). ESI MS: *m/z* 378.3 $[M + Na]^+$, 356.3 $[M + H]^+$.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-hydroxyimino)pyrrolidine-(2*R*)-carboxamide (3).** The ketoester **2** (0.29 g, 0.8 mmol) was mixed with basic NH_2OH solution (3 mL, 5.1 mmol, 1.7 M in methanol, solution prepared as described in Fieser and Fieser, Vol. 1, p 478) and stirred overnight at room temperature. The mixture was then acidified with 1 N HCl and then extracted three times with EtOAc. The combined EtOAc layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by reverse-phase preparative HPLC (60A40B, A, 95% H_2O, 5% acetonitrile, 0.1% formic acid; B, 80% acetonitrile, 20% H_2O; 19 × 300 mm waters SymmetryPrep C_{18} column) to give 100 mg (35% yield) of a white foaming solid 3:4 mixture of *E*:*Z*

isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.93 (t, J = 7.8 Hz, 3H), 1.37−1.48 (m, 2H), 1.64−1.78 (m, 2H), 2.21−2.60 (m, 3H), 3.52−3.60 (m, 1H), 3.68−3.75 (m, 1H), 3.92−4.12 (m, 3H), 7.16 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 9.02 (br s, 1H), 10.86 (s, 1H). ESI MS: m/z 389 $[M + NH_4]^+$, 372 $[M + H]^+$. HRMS: MH^+ calcd for $C_{15}H_{22}N_3O_6S$, 372.1229; found, 372.1228.

Methyl 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxylate (4a). To a solution of ketoester **2** (2.0 g, 5.63 mmol) in dioxane (15 mL), methanol (10 mL), and water (5 mL) were added methoxylamine hydrochloride (1.41 g, 16.9 mmol) and sodium acetate (5.23 g, 63.9 mmol). The mixture was stirred overnight at room temperature and diluted with water. The reaction mixture was extracted three times with EtOAc. The combined EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give 1.9 g (88%) of the desired material as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR ($CDCl_3$): δ 1.02 (t, J = 7.5 Hz, 3H), 1.44−1.59 (m, 2H), 1.76−1.85 (m, 2H), 2.72−2.98 (m, 2H), 3.68 (s, 3H, major isomer), 3.71 (s, 3H, minor isomer), 3.84 (s, 3H), 4.05 (t, J = 6.4 Hz, 2H), 4.12−4.18 (m, 2H), 4.53−4.58 (m, 1H), 6.99 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H). ESI MS: m/z 402 $[M + NH_4]^+$, 385 $[M + H]^+$.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (4).** The methyl ester **5a** (1.0 g, 4.95 mmol) was mixed with NH_2OH (13 mL, 23.4 mmol, 1.7 M in methanol, solution prepared as described in Fieser and Fieser, Vol. 1, p 478) and stirred overnight at room temperature. The mixture was then acidified with 1 N HCl and extracted three times with EtOAc. The combined EtOAc layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified over a column of flash silica eluting with CH_2Cl_2:CH_3OH (95:5) to give 0.69 g (69%) of a white solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.93 (t, J = 7.3 Hz, 3H), 1.38−1.46 (m, 2H), 1.68−1.80 (m, 2H), 2.32−2.59 (m, 2H), 3.70 (s, 1H), 3.77 (s, 2H), 3.88−4.12 (m, 4H), 4.26−4.38 (m, 1H), 7.16 (d, J = 9.0 Hz, 2H), 7.78 (dd, J = 9.0, 3.4 Hz, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: m/z 403 $[M + NH_4]^+$, 386 $[M + H]^+$. Anal. ($C_{16}H_{23}N_3O_6S$) C, H, N.

Methyl 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-morpholineimino)pyrrolidine-(2*R*)-carboxylate (5). To the ketoester **2** (1.5 g, 4.2 mmol) in dioxane (20 mL), methanol (5 mL), and water (5 mL) were added 1-aminomorpholine (0.52 mL, 5.04 mmol) and sodium acetate (3.4 g, 42 mmol). The mixture was stirred overnight at room temperature and diluted with water. The reaction mixture was extracted three times with EtOAc. The combined EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give 1.8 g, (100%) of solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, J = 7.5 Hz, 3H), 1.42−1.58 (m, 2H), 1.78−1.88 (m, 2H), 2.68−2.76 (m, 4H), 2.78−3.01 (m, 2H), 3.69 (s, 3H, major isomer), 3.71 (s, 3H, minor isomer), 3.78−3.82 (m, 4H), 4.00−4.12 (m, 3H), 4.20 (s, 1H), 4.56−4.60 (m, 1H), 6.98−7.01 (m, 2H), 778−7.81 (m, 2H). ESI MS: m/z 440 $[M + H]^+$.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-morpholineimino)pyrrolidine-(2*R*)-carboxamide (6).** The methyl ester (2 g, 4.2 mmol) was mixed with NH_2OH (13 mL, 22 mmol, 1.7 M in methanol, solution prepared as described in Fieser and Fieser, Vol. 1, p 478) and stirred overnight at room temperature. The mixture was neutralized with 1 N HCl to pH ∼ 7 and then concentrated under reduced pressure. The crude product was purified by column eluting with CH_2Cl_2:CH_3OH (95:5) to give 750 mg (43% yield) of a foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.93 (t, J = 7.5 Hz, 3H), 1.38−1.52 (m, 2H), 1.63−1.79 (m, 2H), 2.39−2.63 (m, 6H), 3.56−3.70 (m, 4H), 3.92−4.12 (m, 4H), 4.20−4.37 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 9.0 Hz, 2H), 9.01 (s, 1H), 10.83 (s, 1H). ESI MS: m/z 479 $[M + K]^+$, 441 $[M + H]^+$. Anal. ($C_{19}H_{28}N_4O_6S\cdot0.25H_2O$) C, H, N.

Methyl 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*N*-methoxyamino)pyrrolidine-(2*R*)-carboxylate (7a). To a solution of oxime **4a** (0.95 g, 2.47 mmol) in methanol (10 mL) was added sodium cyanoborohydride (2.2 g, 35 mmol) followed by slow addition of concentrated HCl (1 mL). The mixture was stirred overnight at room temperature, and then the pH was adjusted to 10 with 1 N NaOH. The reaction mixture was extracted three times with EtOAc. The combined EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give an oil which was purified by column eluting with EtOAc/hexane (1:1) to give 0.66 g (71% yield) of the desired product as a 2:1 mixture of α:β epimers (unassigned). ^{1}H NMR ($CDCl_3$): δ 1.00 (td, J = 7.3, 1.3 Hz, 3H), 1.40−1.54 (m, 2H), 1.73−1.81 (m, 2H), 2.02−2.19 (m, 2H), 3.28−3.37 (m, 2H), 3.41−3.47 (m, 3H), 3.49−3.63 (m, 1H), 3.72 (s, 1H), 3.75 (s, 2H), 4.01 (t, J = 6.4 Hz, 2H), 4.21−4.33 (m, 1H), 5.17 (d, J = 7.1 Hz, 1/3H), 5.76 (J = 9.0 Hz, 2/3H), 6.97 (d, J = 9.2 Hz, 2H), 7.78 (d, J = 9.2 Hz, 2H). ESI MS: m/z 387 $[M + H]^+$.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*N*-methoxyamino)pyrrolidine-(2*R*)-carboxamide (7).** The methyl ester **7a** (0.3 g, 0.78 mmol) was treated with NH_2OH (6.4 mL, 10.8 mmol, 1.7 M in methanol, solution prepared as described in Fieser and Fieser, Vol. 1, p 478) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and purified by column eluting with CH_2Cl_2:CH_3OH (95:5) to give 0.17 g (56% yield) of a white solid as a 2:1 mixture of α:β epimers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.97 (t, J = 7.2 Hz, 3H), 1.39−1.47 (m, 2H), 1.63−1.79 (m, 3H), 1.81−2.00 (m, 1H), 2.98−3.15 (m, 2H), 3.22−3.42 (m, 3H), 3.81−3.93 (m, 1H), 4.04−4.14 (m, 3H), 6.38 (d, J = 6.7 Hz, 1/3H), 6.84 (d, J = 9.0 Hz, 2/3H), 7.08−7.19 (m, 2H), 7.73−7.81 (m, 2H), 8.97 (s, 1/3H), 9.02 (s, 2/3H), 10.69 (s, 1/3H), 10.79 (s, 2/3H). ESI MS: m/z 388 $[M + H]^+$. HRMS: MH^+ calcd for $C_{16}H_{26}N_3O_6S$, 388.1542; found, 388.1559.

(1*N*)-4-Methoxyphenylsulfonyl-(2*R*)-carbomethoxy-(4*R*)-hydroxypyrrolidine (8a). *cis*-Hydroxy-D-proline **1** (50 g, 0.38 mol) was dissolved in water:dioxane (1:1, 300 mL) with triethylamine (135 mL, 0.96 mol). 4-Methoxyphenylsulfonyl chloride (87 g, 0.42 mol) was added along with 4-(dimethylamino)pyridine (4.6 g, 0.038 mol), and the mixture was stirred 14 h at room temperature. The mixture was then concentrated and diluted with EtOAc. Layers were separated, and the organic layer was washed 2× with 1 N HCl, 1× with brine, dried over $MgSO_4$, filtered, and evaporated to give 83 g of solid material which was dissolved in MeOH (500 mL). Thionyl chloride (50 mL) was added dropwise and the resulting mixture stirred for 14 h. The mixture was then evaparated to dryness and triturated with $CHCl_3$ to give 85 g (69%) of white solid which was sufficiently pure to carry forward without purification. ^{1}H NMR ($CDCl_3$): δ 2.07 (ddd, J = 14.1, 3.8, 1.6 Hz, 1H), 2.16 (ddd, J = 14.1, 9.5, 4.4 Hz, 1H), 3.31 (dd, J = 10.3, 4.2 Hz, 1H), 3.48−3.54 (m, 2H), 3.76 (s, 3H), 3.89 (s, 3H), 4.28−4.34 (m, 2H), 6.98 (ddd, J = 9.0, 2.5, 2.5 Hz, 2H), 7.79 (ddd, J = 9.1, 2.6, 2.6 Hz, 2H). CI^+ MS: m/z 316 $[M + H]^+$, 256, 146, 114.

Methyl 1*N*-(4-Methoxyphenylsulfonyl)-4-oxo-pyrrolidine-(2*R*)-carboxylate (8). An 8 M batch of Jones reagent was prepared. The alcohol **8a** (10.0 g, 31.7 mmol) is dissolved in 175 mL of acetone and cooled to 0 °C. Jones reagent was added until the solution remains an orange color, and the mixture is stirred at room temperature for 14 h. 2-Propanol was added to the solution to quench the excess chromium reagent, and the resulting solid was filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was dissolved in methylene chloride and washed with water. The resulting solution was dried over magnesium sulfate and concentrated under reduced pressure. Purification of the product by chromatography on silica gel using EtOAc:hexane (1:1) provided the desired ketone. ^{1}H NMR ($CDCl_3$): δ 2.57 (dd, J = 18.3, 3.3 Hz, 1H), 2.80 (dd, J = 18.3, 9.2 Hz, 1H), 3.66 (s, 3H), 3.80 (s, 1H), 3.82 (s, 1H), 3.71 (s, 3H), 4.78 (dd, J = 8.8, 3.3 Hz, 1H), 7.02 (br d, J = 8.8 Hz, 2H), 7.80 (br d, J = 8.8 Hz, 2H). ESI MS: m/z 374 $[M^+ + H]^+$.

Methyl 1*N*-(4-Methoxyphenyl)sulfonyl-4-methylene-pyrrolidine-(2*R*)-carboxylate (10). To a solution of meth-

yltriphenylphosphonium bromide (1.75 g, 4.78 mmol) in 10 mL of anhydrous THF at 0 °C under argon was added lithium bis-(trimethylsilyl)amide (5.74 mL, 5.74 mmol, 1.0 M solution in THF) dropwise and stirred for 15 min. Then, a solution of ketone **8** (1.5 g, 4.78 mmol) in THF (25 mL) was added slowly. The mixture was stirred overnight at room temperature and diluted with ammonium chloride. The reaction mixture was extracted three times with EtOAc. The combined EtOAc layer was washed with 1 N HCl, water, aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to an oil which was purified by column chromatography eluting with EtOAc:hexane (3:7) to give 0.35 g (25%) of the desired product. ^{1}H NMR ($CDCl_3$): δ 2.58−2.87 (m, 2H), 3.63 (s, 3H), 3.83 (s, 3H), 4.01−4.15 (m, 2H), 4.43−4.59 (m, 1H), 5.01 (d, J = 10.3 Hz, 2H), 6.99 (dd, J = 9.0, 2.0, Hz, 2H), 7.82 (dd, J = 9.0, 2.0 Hz, 2H). ESI MS: m/z 329 $[M + NH_4]^+$, 312 $[M + H]^+$.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-methylene-pyrrolidine-(2*R*)-carboxamide (11).** The methyl ester **10** (0.26 g, 0.83 mmol) was mixed with NH_2OH (3.7 mL, 6.64 mmol, 1.7 M in methanol, solution prepared as described in Fieser and Fieser, Vol. 1, p 478) and stirred overnight at room temperature. Neutralized with 1 N HCl, the mixture was extracted three times with EtOAc. The combined EtOAc layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column eluting with CH_2Cl_2:CH_3OH (95:5) to give 0.18 g (70% yield) of the desired product as a white solid. ^{1}H NMR (DMSO-d_6): δ 2.26−2.42 (m, 2H), 3.83−3.90 (m, 1H), 3.87 (s, 3H), 3.95−4.02 (m, 2H), 4.08−4.16 (m, 1H), 4.94 (d, J = 10.3 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 10.86 (s, 1H). ESI MS: m/z 330 $[M + NH_4]^+$, 313 $[M + H]^+$. Anal. ($C_{13}H_{16}N_3O_5S\cdot 0.75H_2O$) C, H, N.

(1*N*)-*tert*-Butoxycarbonyl-(2*R*)-carbomethoxy-(4*R*)-hydroxypyrrolidine (12a). *cis*-Hydroxy-D-proline methyl ester hydrochloride (40 g, 220 mmol, the methyl ester was obtained from methanolic HCl treatment of the relative acid which was obtained from Aldrich) was taken in 205 mL of acetone:water (3:2) in the presence of 100 mL of Et_3N and 1 g of 4-(dimethylamino)pyridine, and the mixture was treated in portions with di-*tert*-butyl dicarbonate (53 g, 243 mmol). After warming slightly, the mixture was cooled back to room temperature and stirred for 18 h. The mixture was reduced to $\sim^1/_3$ of its volume and then partitioned between EtOAc and dilute NaH_2PO_4. The organic layer was washed 1× with dilute NaH_2PO_4 and 1× with brine, dried over $MgSO_4$, filtered, and evaporated to give 68 g of white solid which was carried forward without purification. ^{1}H NMR ($CDCl_3$): *This compound appears in the ^{1}H NMR spectrum as a distinct pair of rotomers.* δ [1.44 (s), 1.48 (s)] 9H, 2.06−2.16 (m, 1H), 2.27−2.42 (m, 1H), 3.50−3.75 (m, 2H), [3.80 (s), 3.81 (s)] 1H, 4.29−4.42 (m, 2H). ESI MS: m/z 262.9 $[M + NH_4]^+$, 245.9 $[M + H]^+$, 145.8 $[M - Boc + H]^+$.

Boc-Ketopyrrolidine Methyl Ester (12). The starting alcohol **12a** (16.4 g, 66.9 mmol) was oxidized as described for compound **2**. The crude syrup was chromatographed over flash silica with hexane:Et_2O (4:1, 1:2) to give 11.1 g (68%) of white solid. ^{1}H NMR ($CDCl_3$): *The compound appears as a pair of distinct rotomers.* δ 1.46 (br s, 9H), 2.58 (dd, J = 17.0, 1.9 Hz, 1H), 2.56−3.03 (m, 1H), 3.77 (s, 3H), 3.86−3.93 (m, 2H), [4.26−4.41 (m), 4.67 (dd, J = 17.6, 9.6 Hz)] 1H.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-hydroxyimino)pyrrolidine-(2*R*)-carboxamide (13).** The title compound was prepared as described for compound **3** and gave a white cotton like solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.26−2.62 (m, 3H), 3.89 (s, 3H), 3.92−4.13 (m, 2H), 4.24−4.39 (m, 1H), 7.12−7.20 (m, 2H), 7.78−7.86 (m, 2H), 9.02 (s, 1H), 10.87 (s, 1H). ESI MS: m/z 347 $[M + NH_4]^+$, 330 $[M + H]^+$. Anal. ($C_{12}H_{15}N_3O_6S\cdot 0.5H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (14).** The title compound was prepared as described for compound **4** and gave a white solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.33−2.62 (m, 2H), 1.20−1.38 (m, 6H), 3.72 (s, 3H, minor isomer), 3.76 (s, 3H, major isomer), 3.86 (s, 3H), 3.88−4.13 (m, 2H), 4.26−4.39 (m, 1H), 7.16 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: m/z 366 $[M + Na]^+$, 344 $[M + H]^+$. Anal. ($C_{13}H_{17}N_3O_6S\cdot 0.75H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-Ethoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (15).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.28−2.01 (m, 3H), 2.24−2.59 (m, 2H), 3.68−3.75 (m, 3H), 3.93−4.18 (m, 4H), 4.26−4.35 (m, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.74 (d, J = 9.0 Hz, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: m/z 375 $[M + NH_4]^+$, 358 $[M + H]^+$. Anal. ($C_{14}H_{19}N_3O_6S\cdot 0.25H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-Propoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (16).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.99 (t, J = 7.3 Hz, 3H), 1.64−1.79 (m, 2H), 2.25−2.59 (m, 2H), 3.72 (d, J = 9.5 Hz, 3H), 3.83−4.08 (m, 4H), 4.26−4.35 (m, 1H), 7.09 (d, J = 9.0 Hz, 2H), 7.69−7.99 (m, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: m/z 389 $[M + NH_4]^+$, 372 $[M + H]^+$. Anal. ($C_{15}H_{21}N_3O_6S\cdot 0.25H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-(2-Methoxyethoxy)phenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)-pyrrolidine-(2*R*)-carboxamide (17).** The title compound was prepared as described for compound **4** and gave a white solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.37−2.60 (m, 2H), 3.63−3.72 (m, 4H), 3.74 (s, 1H), 3.78 (s, 2H), 3.89−4.08 (m, 3H), 4.15−4.23 (m, 2H), 4.28−4.37 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.78 (dd, J = 9.0 Hz, 2H), 7.78 (dd, J = 9.0, 1.7 Hz, 2H), 9.01 (s, 1H), 10.88 (s, 1H). ESI MS: m/z 405 $[M + NH_4]^+$, 388 $[M + H]^+$. HRMS: MH^+ calcd for $C_{15}H_{22}N_3O_7S$, 388.1178; found, 388.1184.

***N*-Hydroxy 1*N*-(4-Phenoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (18).** The title compound was prepared as described for compound **4**. The crude product was purified by reverse-phase preparative HPLC (55A45B, A, 95% H_2O, 5% acetonitrile, 0.1% formic acid; B, 80% acetonitrile, 20% H_2O; 19 × 300 mm waters SymmetryPrep C_{18} column) to give 250 mg (44% yield) of a white foaming solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.41−2.77 (m, 2H), 3.68−3.74 (m, 3H), 3.91−4.12 (m, 2H), 4.30−4.38 (m, 1H), 7.06−7.19 (m, 4H), 7.21−7.34 (m, 1H), 7.43−7.52 (m, 2H), 7.77−8.05 (m, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: m/z 423 $[M + NH_4]^+$, 406 $[M + H]^+$. Anal. ($C_{18}H_{19}N_3O_6S$) C, H, N.

***N*-Hydroxy 1*N*-[4-(4-Fluorophenoxy)phenyl]sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)-pyrrolidine-(2*R*)-carboxamide (19).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.41−2.58 (m, 1H), 2.59−2.74 (m, 1H), 3.69−3.77 (m, 3H), 3.92−4.10 (m, 2H), 4.22−4.36 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.19−7.38 (m, 4H), 7.78−7.84 (m, 2H), 9.02 (s, 1H), 10.87 (s, 1H). ESI MS: m/z 441 $[M + NH_4]^+$, 424 $[M + H]^+$. Anal. ($C_{18}H_{18}N_3FO_6S$) C, H, N.

***N*-Hydroxy 1*N*-(4-Pyridyloxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-methoxyimino)pyrrolidine-(2*R*)-carboxamide (20).** The title compound was prepared as described for compound **4**. The crude product was purified by reverse-phase preparative HPLC (90A10B, A, 95% H_2O, 5% acetonitrile, 0.1% formic acid; B, 80% acetonitrile, 20% H_2O; 19 × 300 mm waters SymmetryPrep C_{18} column) to give a white foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.41−2.58 (m, 1H), 2.68−2.79 (m, 1H), 3.61−3.79 (m, 3H), 3.97−4.16 (m, 2H), 4.29−4.39 (m, 1H), 7.01−7.12 (m, 2H), 7.28−7.39 (m, 2H), 7.84−7.79 (m, 2H), 8.26 (s, 1H), 8.50−8.59 (m, 2H), 10.87 (s, 1H). ESI MS: m/z 407 $[M + H]^+$. Anal. ($C_{17}H_{18}N_4O_6S\cdot H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-ethoxyimino)pyrrolidine-(2*R*)-carboxamide (21).** The title compound was prepared as described for compound **4**. The crude product was purified by reverse-phase preparative HPLC (90A10B, A, 95% H_2O, 5% acetonitrile, 0.1% formic acid;

B, 80% acetonitrile, 20% H_2O; 19 × 300 mm waters SymmetryPrep C_{18} column) to give a white foaming solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.93 (t, J = 7.5 Hz, 3H), 1.02−1.18 (m, 3H), 1.39−1.52 (m, 2H), 1.66−1.81 (m, 2H), 2.27−2.62 (m, 3H), 3.87−4.10 (m, 5H), 4.28−4.39 (m, 1H), 7.10 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 9.0 Hz, 2H), 9.04 (s, 1H), 10.86 (s, 1H). ESI MS: *m*/*z* 417 [M + Na]$^+$, 400 [M + H]$^+$. Anal. ($C_{17}H_{25}N_3O_6S\cdot0.25H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-*tert*-butoxyimino)pyrrolidine-(2*R*)-carboxamide (22).** The title compound was prepared as described for compound **4**. The crude product was purified by reverse-phase preparative HPLC (90A10B, A, 95% H_2O, 5% acetonitrile, 0.1% formic acid; B, 80% acetonitrile, 20% H_2O; 19 × 300 mm waters SymmetryPrep C_{18} column) to give a white foaming solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.19 (s, 9H), 2.26−2.52 (m, 2H), 3.82 (s, 3H), 3.99−4.07 (m, 2H), 4.32−4.40 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: *m*/*z* 403 [M + NH$_4$]$^+$, 386 [M + H]$^+$. Anal. ($C_{16}H_{23}N_3O_6S\cdot0.75H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-*tert*-butoxyimino)pyrrolidine-(2*R*)-carboxamide (23).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 1:1 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.95 (t, J = 7.5 Hz, 3H), 1.14 (s, 4.5H), 1.19 (s, 4.5H), 1.38−1.52 (m, 2H), 1.68−1.80 (m, 2H), 2.26−2.64 (m, 2H), 3.86−4.12 (m, 4H), 4.28−4.40 (m, 1H), 7.12 (dd, J = 9.0, 3.6 Hz, 2H), 7.70−7.82 (m, 2H), 9.05 (s, 1H), 10.89 (s, 1H). ESI MS: *m*/*z* 450.1 [M + Na]$^+$, 445.1 [M + NH$_4$]$^+$, 428.1 [M + H]$^+$. Anal. ($C_{19}H_{29}N_3O_6S$) C, H, N.

***N*-Hydroxy 1*N*-(4-Pyridyloxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-*tert*-butoxyimino)-pyrrolidine-(2*R*)-carboxamide (24).** The title compound was prepared as described for compound **4** and gave a white solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.23 (s, 9H), 2.42−2.59 (m, 1H), 2.66−2.81 (m, 1H), 3.98−4.17 (m, 2H), 4.32−4.41 (m, 1H), 6.99−7.12 (m, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.86−8.01 (m, 2H), 8.50−8.61 (m, 2H), 9.06 (s, 1H), 10,86 (s, 1H). ESI MS: *m*/*z* 449 [M + H]$^+$. Anal. ($C_{20}H_{24}N_4O_6S\cdot0.25H_2O$) C, H, N.

***N*-Hydroxy 1*N*-[4-(4-Fluorophenoxy)phenyl]sulfonyl-4-(*Z*,*E*-*N*-*tert*-butoxyimino)-pyrrolidine-(2*R*)-carboxamide (25).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 1:4 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.21 (s, 9H), 2.40−2.52 (m, 1H), 2.60−2.72 (m, 1H), 3.88−4.12 (m, 2H), 4.27−4.36 (m, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.19−7.25 (m, 2H), 7.28−7.39 (m, 2H), 7.81 (d, J = 9.0 Hz, 2H), 9.02 (s, 1H), 10.86 (s, 1H). ESI MS: *m*/*z* 483 [M + NH$_4$]$^+$, 466 [M + H]$^+$. Anal. ($C_{21}H_{24}N_3O_6FS\cdot0.25H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-isobutoxyimino)pyrrolidine-(2*R*)-carboxamide (26).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 1:2 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.76−0.88 (m, 6H), 0.95 (t, J = 7.4 Hz, 3H), 1.40−1.51 (m, 2H), 1.66−1.87 (m, 3H), 2.30−2.60 (m, 2H), 3.62−3.75 (m, 2H), 3.91−4.10 (m, 4H), 4.28−4.39 (m, 1H), 7.12 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 9.0 Hz, 2H), 9.07 (s, 1H), 10.86 (s, 1H). ESI MS: *m*/*z* 445 [M + NH$_4$]$^+$, 428 [M + H]$^+$. Anal. ($C_{19}H_{29}N_3O_6S$) C, H, N.

***N*-Hydroxy 1*N*-(4-Pyridyloxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-phenoxyimino)pyrrolidine-(2*R*)-carboxamide (27).** The title compound was prepared as described for compound **4** and gave a white solid as a 3:4 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 2.68−2.98 (m, 1H), 2.82−3.01 (m, 1H), 3.22−3.40 (m, 2H), 3.79−3.86 (m, 2H), 6.82−6.88 (m, 1H), 6.92−7.03 (m, 2H), 7.19 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H), 7.36−7.41 (m, 3H), 7.60 (s, 3H), 7.68−7.78 (m, 2H), 10.62 (s, 2H). ESI MS: *m*/*z* 469 [M + H]$^+$. HRMS: MH$^+$ calcd for $C_{22}H_{21}N_4O_6S$, 469.1182; found, 469.1177.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-benzyloxyimino)pyrrolidine-(2*R*)-carboxamide (28).** The title compound was prepared as described for compound **4** and gave a white foaming solid as a 2:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.96 (t, J = 7.3 Hz, 3H), 1.40−1.52 (m, 2H), 1.65−1.80 (m, 2H), 2.43−2.63 (m, 2H), 3.98−4.08 (m, 4H), 4.23−4.36 (m, 1H), 5.00 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 9.0 Hz, 2H), 7.09−7.39 (m, 5H), 7.77 (d, J = 9.0 Hz, 2H), 9.04 (s, 1H), 10.89 (s, 1H). ESI MS: *m*/*z* 479 [M + NH$_4$]$^+$, 462 [M + H]$^+$. HRMS: MH$^+$ calcd for $C_{22}H_{28}N_3O_6S$, 462.1699; found, 462.1683.

***N*-Hydroxy 1*N*-(4-*n*-Butoxyphenyl)sulfonyl-4-(*Z*,*E*-*N*-piperidineimino)pyrrolidine-(2*R*)-carboxamide (29).** The title compound was prepared as described for compound **7** and give a solid as a 4:3 mixture of *E*:*Z* isomers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.92 (t, J = 7.5 Hz, 3H), 1.24−1.60 (m, 9H), 1.62−1.78 (m, 2H), 2.26−2.60 (m, 5H), 3.82−4.10 (m, 4H), 4.18−4.34 (m, 1H), 7.08 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 9.0 Hz, 2H), 9.00 (s, 1H), 10.86 (s, 1H). ESI MS: *m*/*z* 439 [M + H]$^+$. Anal. ($C_{20}H_{30}N_4O_5S\cdot0.5H_2O$) C, H, N.

Methyl 1*N*-*tert*-Butoxycarbonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxylate (30a). An oven dried/argon cooled flask is charged with methyltriphenylphosphonium bromide (1.29 g, 3.61 mmol) and 20 mL of dry benzene. Over a period of 30 s, sodium *tert*-amylate (2.4 mL, 1.65 M in toluene, 3.95 mmol) was added via syringe, and the mixture was allowed to stir at room temperature under argon for 30 min. A solution of keto ester **8** (0.6 g, 2.47 mmol) in dry benzene was then added in one portion via syringe, and the mixture was allowed to stir for an additional 3 h. The mixture was then quenched with 15 mL of 10% NH_4Cl, and the layers were separated. The aqueous layer was extracted once with diethyl ether, and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give 1.29 g of very viscous oil with crystals. The crude product was dissolved in 5 mL of hot hexane/EtOAc and filtered through 10% deactivated silica eluting with EtOAc: hexane (1:3). The filtrate was evaporated to dryness, the residue was dissolved in 1.5 mL of EtOAc, and 5 mL of hexane was added. The crystallized triphenylphosphine oxide is filtered off, and after evaporation, the filtrate furnishes 470 mg (71%) of the desired material in the form of a yellowish oil containing about 5% of triphenylphosphine oxide impurity. ^{1}H NMR ($CDCl_3$): *The compound appears in the spectrum as two distinct rotomers in a 2:3 ratio.* δ [1.44 (s), 1.49 (s)] 9H, 2.58−2.69 (m, 1H), 2.89−3.06 (m, 1H), 3.74 (s, 3H), 4.04−4.13 (m, 2H), [4.42 (dd, J = 9.2, 3.5 Hz), 4.51 (dd, J = 9.5, 2.8 Hz)] 1H, 4.98−5.06 (m, 2H). ESI MS: *m*/*z* 259.0 [M + NH$_4$]$^+$, 242.0 [M + H]$^+$, 185.8, 141.

Methyl 1*N*-(4-*n*-Propyloxyphenyl)-sulfonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxylate (30b). The exomethylene compound **30a** (494 mg, 2.04 mmol) was taken in 25 mL of methylene chloride and treated with 2 mL of trifluoroacetic acid. The mixture was stirred for 1 h at room temperature, evaporated to dryness, and triturated once with chloroform. The residual oil was taken in 25 mL of methylene chloride in the presence of 3 mL of Et_3N and treated with *n*-propoxybenzenesulfonyl chloride. The mixture was stirred for 18 h and then partitioned between $CHCl_3$ and 1 N HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was adsorbed onto silica and then eluted through a column of flash silica with hexane:EtOAc (8:2 to 5:5) to give 334 mg (48%) of clear viscous oil. ^{1}H NMR ($CDCl_3$): δ 1.07 (t, J = 7.4 Hz, 3H), 1.79−1.92 (m, 2H), 2.60 (m, 1H), 2.73−2.85 (m, 1H), 3.69 (s, 3H), 4.00 (t, J = 6.6 Hz, 2H), 4.02 (br s, 2H), 4.46 (dd, J = 8.8, 3.9 Hz, 1H), 4.97−5.01 (m, 1H), 6.99 (ddd, J = 9.2, 2.9, 2.2 Hz, 2H), 7.80 (ddd, J = 9.2, 2.9, 2.2 Hz, 2H). ESI MS: *m*/*z* (rel intensity) 340.0 [M + NH$_4$]$^+$, 357.0 [M + H]$^+$.

***N*-Hydroxyl 1*N*-(4-*n*-Propoxyphenyl)-sulfonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxamide (30).** The compound was prepared from methyl ester **30b** (317 mg, 0.93 mmol) as described for compound **11** to give 168 mg of white solid. ^{1}H NMR (DMSO-d_6): δ 1.01 (t, J = 7.3 Hz, 3H), 1.72−1.86 (m, 2H), 2.30−2.49 (m, 2H), 3.89 (d, J = 14.5 Hz, 1H), 4.02 (d, J = 14.5 Hz, 1H), 4.06 (t, J = 6.4 Hz, 2H), 4.15 (dd, J = 8.4, 3.8 Hz, 1H), 4.91 (s, 1H), 4.95 (s, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 8.99 (s, 1H), 10.56 (s, 1H).

ESI MS: *m/z* 358.0 $[M + NH_4]^+$, 341.0 $[M + H]^+$, 168.9. Anal. ($C_{15}H_{20}N_2O_5S \cdot 0.1H_2O$) C, H, N.

***N*-Hydroxyl 1*N*-(4-*n*-Butoxyphenyl)-sulfonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxamide (31).** The compound was prepared as described for compound **30** to give a white solid. ^{1}H NMR (DMSO-d_6): δ 0.2.30−2.49 (m, 2H), 3.35 (s, 3H), 3.68−3.75 (m, 2H), 3.89 (d, J = 14.7 Hz, 1H), 4.03 (d, J = 14.8 Hz, 1H), 4.15 (dd, J = 8.4, 3.8 Hz, 1H), 4.20−4.27 (m, 2H), 4.92 (s, 1H), 4.95 (s, 1H), 7.16 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 9.00 (s, 1H), 10.77 (s, 1H). ESI MS: *m/z* 372.0 $[M + NH_4]^+$, 355.0 $[M + H]^+$, 168.9. Anal. ($C_{16}H_{22}N_2O_5S \cdot 0.3H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-(2-Methoxyethoxy)phenyl)sulfonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxamide (32).** The compound was prepared as described for compound **30** to give a white solid. ^{1}H NMR (DMSO-d_6): δ 2.30−2.49 (m, 2H), 3.35 (s, 3H), 3.68−3.75 (m, 2H), 3.89 (d, J = 14.7 Hz, 1H), 4.03 (d, J = 14.8 Hz, 1H), 4.15 (dd, J = 8.4, 3.8 Hz, 1H), 4.20−4.27 (m, 2H), 4.92 (s, 1H), 4.95 (s, 1H), 7.16 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 9.00 (s, 1H), 10.77 (s, 1H). ESI MS: *m/z* 374.0 $[M + NH_4]^+$, 357.0 $[M + H]^+$, 168.9. Anal. ($C_{15}H_{20}N_2O_6S \cdot 0.5H_2O$) C, H, N.

***N*-Hydroxyl 1*N*-(4-*n*-Phenoxyphenyl)-sulfonyl-4-exomethylene-pyrrolidine-(2*R*)-carboxamide (33).** The compound was prepared as described for compound **30** to give a white solid. ^{1}H NMR (DMSO-d_6): δ 2.45 (d, J = 5.68 Hz, 2H), 3.91 (d, J = 14.5 Hz, 1H), 4.04 (d, J = 14.5 Hz, 1H), 4.16 (t, J = 6.3 Hz, 1H), 4.96 (s, 1H), 4.96 (s, 1H), 7.13−7.22 (m, 4H), 7.30 (ddd, J = 7.3, 7.3, 0.9 Hz, 1H), 7.51 (dd, J = 7.9 Hz, 2H), 7.87 (br d, J = 8.8 Hz, 2H), 8.99 (br s, 1H), 10.77 (s, 1H). ESI MS: *m/z* 392.0 $[M + NH_4]^+$, 375.0 $[M + H]^+$, 168.9. Anal. ($C_{18}H_{18}N_2O_5S \cdot 0.2H_2O$) C, H, N.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-(*N*-methoxyamino)pyrrolidine-(2*R*)-carboxamide (34).** The title compound was prepared as described for compound **7** to give a white solid as a 2:1 mixture of α:β epimers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.64−2.03 (m, 2H), 2.98−3.19 (m, 1H), 3.22−3.39 (m, 1H), 3.38 (s, 3H), 3.40−4.60 (m, 1H), 3.81−3.97 (m, 1H), 3.85 (s, 3H), 6.38−6.40 (m, 1/3H), 6.80−6.87 (d, J = 8.9 Hz, 2/3H), 7.08−7.18 (m, 2H), 7.73−7.81 (m, 2H), 8.93 (s, 1/3H), 9.04 (s, 2/3H), 10.68 (s, 1/3H), 10.79 (s, 2/3H). ESI MS: *m/z* 346 [M + H]. Anal. ($C_{13}H_{19}N_3O_6S$) C, H, N.

***N*-Hydroxy 1*N*-(4-(2-Methoxyethoxy)phenyl)sulfonyl-4-(*N*-methoxyamino)-pyrrolidine-(2*R*)-carboxamide (35).** The title compound was prepared as described for compound **7** to give a white solid as a 2:1 mixture of α:β epimers (unassigned). ^{1}H NMR (DMSO-d_6): δ 0.99 (t, J = 7.51 Hz, 3H), 1.40−1.55 (m, 2H), 1.70−1.81 (m, 2H), 2.30−2.49 (m, 2H), 3.89 (d, J = 14.8 Hz, 1H), 4.03 (d, J = 14.7 Hz, 1H), 4.07−4.18 (m, 3H), 4.92 (s, 1H), 4.95 (s, 1H), 7.16 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 9.00 (s, 1H), 10.75 (s, 1H). ESI MS: *m/z* 390 $[M + H]^+$. HRMS: MH^+ calcd for $C_{15}H_{25}N_3O_7S$, 390.1335; found, 390.1348.

***N*-Hydroxy 1*N*-(4-Phenoxyphenyl)sulfonyl-4-(*N*-methoxyamino)pyrrolidine-(2*R*)-carboxamide (36).** The title compound was prepared as described for compound **7** to give a white solid as a 2:1 mixture of α:β epimers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.63−1.78 (m, 1H), 1.97−2.07 (m, 1H), 3.13−3.21 (m, 1H), 3.27−3.35 (m, 5H), 3.86−3.93 (m, 1H), 6.81−6.92 (m, 1H), 7.12−7.21 (m, 4H), 7.22−7.39 (m, 1H), 7.43−7.53 (m, 2H), 7.82 (d, J = 9.0 Hz, 2H), 9.08 (s, 1H), 10.76 (s, 1H). ESI MS: *m/z* 408 $[M + H]^+$. Anal. ($C_{18}H_{21}N_3O_6S$) C, H, N.

***N*-Hydroxy 1*N*-(4-Methoxyphenyl)sulfonyl-4-(*N*-*tert*-butoxyamino)pyrrolidine-(2*R*)-carboxamide (37).** The title compound was prepared as described for compound **7** to give a white solid as a 3:4 mixture of α:β epimers (unassigned). ^{1}H NMR (DMSO-d_6): δ 1.02 (s, 4H), 1.19 (s, 5H), 1.82−2.27 (m, 3H), 3.08−3.20 (m, 1H), 3.39−3.67 (m, 2H), 3.84 (s, 3H), 4.14−4.21 (m, 1H), 7.02 (d, J = 9.0 Hz, 2H), 7,82 (d, J = 9.0 Hz, 2H), 9.52−9.79 (m, 1H). ESI MS: *m/z* 388 $[M + H]^+$. Anal. ($C_{16}H_{25}N_3O_6S$) C, H, N.

Expression and Purification of Human Recombinant Truncated MMP-1. Briefly, DNA sequence coding for Val82-Pro249 of proMMP-1 was amplified by polymerase chain reaction from a commercially available plasmid, p35-1 (ATCC, Rockville, MD; Templeton, N. S.; et al. (1990) *Cancer Res.* **1990**, *50*, 5431−5437) encoding human interstitial MMP-1. The PCR fragment was ligated into the expression vector, pET-11a (Novagen, Madison, WI), and expressed in *E. coli* BL21(DE3) cells. The protein was solubilized from inclusion bodies in 6 M urea, 0.15 M NaCl, pH 7.5, refolded in 50 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.1 mM zinc acetate, and then purified to homogeneity over a hydroxamic acid inhibitor affinity column as previously described (Moore, W. A.; Spilburg, C. A. *Biochemistry* **1986**, *25*, 5189−5195). N-Terminal sequence analysis confirmed the presence of three N-terminal sequences of Val-Leu-Thr-Glu-Gly-Asn, Met-Val-Leu-Thr-Glu-Gly-Asn, and Leu-Thr-Glu-Gly-Asn (minor).

Expression and Purification of Human Recombinant proMMP-2. A partial cDNA clone for MMP-2 known as K-121 (Tryggvason, K. *J. Biol. Chem.* **1990**, *265*, 11077−11082) was obtained from ATCC and subcloned into the pBlueScript SK^- (pBS) plasmid. Sanger dideoxy sequencing revealed that the first 134 bp of the coding sequence were missing from the 5′ end of K-121. To restore the missing sequence, two overlapping 90+-mer oligonucleotides were designed and synthesized. These, along with a 3′ antisense oligonucleotide, were used as primers to the K-121 template in a series of polymerase chain reaction (PCR) experiments to synthesize a full-length MMP-2 cDNA. The PCR product was then subcloned into pBS. To express MMP-2 in the mammalian CHO D^- cell system, it was first subcloned into the mammalian expression vector pJT1 (J. Ting, CRD), which contains the DHFR gene. Recombinant MMP-2/pJT1 was Polybrene transfected into CHO D^- cells. Clonal cell populations were isolated and screened for production of MMP-2 mRNA using specific oligonucleotide primers and reverse-transcription PCR. Ten clones producing the highest levels of MMP-2 mRNA were selected, and the DHFR/MMP-2 construct was amplified by gradually increasing the media methotrexate (MTX, a DHFR inhibitor) concentration. Clonal selection was further narrowed after assessing MMP bioactivity in an MMP fluorescence assay (M. Anastasio, CRD) and on zymogram gels. Those clones showing activity were tested for MMP-2 protein production by sequential Edman degradation protein sequencing (F. Wang, P&GP Cell & Molecular Biology Core) and Western blot. On the basis of all results, one clone was expanded for growth in roller bottles. Approximately 9 L of serum-free conditioned media with an MMP-2 concentration of ∼ 35 mG/L was generated. ProMMP-2 was purified from conditioned media as previously described (Crabbe, T. et al. *Eur. J. Biochem.* **1993**, *218*, 431−438) with the following modifications. Conditioned serum free media was reduced in volume with an Amicon S1Y30 spiral-wound cartridge prior to chromatography on a gelatin-Sepharose 4B column equilibrated in 25 mM Tris/HCl, 30 mM NaCl, 10 mM $CaCl_2$, pH 7.5 (TNC buffer). The column was washed with equilibration buffer before elution of the bound protein by TNC buffer containing 1 M NaCl followed by 1 M NaCl and 10% (by volume) dimethyl sulfoxide (DMSO) in TNC buffer. Pro-MMP-2 fractions were concentrated in an Amicon ultrafiltration cell with a YM30 membrane and diafiltered against 25 mM MES/NaOH, 30 mM NaCl, 10 mM $CaCl_2$, pH 6.0 (MNC buffer). The concentrate was then chromatographed over a second gelatin-Sepharose 4B column equilibrated in MNC buffer, washed with equilibration buffer to remove nonbinding proteins, and eluted with MNC buffer containing 0.3 M NaCl and 10% (by volume) DMSO. Elution fractions containing progelatinase A were pooled, diluted, and loaded onto an S-Sepharose Fast Flow column equilibrated in MNC buffer and eluted with MNC buffer containing 0.3 M NaCl. The concentrated fractions of purified proMMP-2 were combined and stored in MNC buffer containing 0.3 M NaCl at −70 °C. N-Terminal sequence, amino acid, and mass spectrophotometric analysis confirmed the identity of the purified protein with the expected sequence for progelatinase A.

Expression and Purification of Human Recombinant Truncated MMP-3. Briefly, DNA sequence coding for Ala^{-1}-

Thr^{255} of proMMP-3 was amplified by polymerase chain reaction from a recombinant plasmid encoding human synovial preproMMP-3 kindly provided by Dr. Hideaki Nagase (University of Kansas Medical Center, Kansas City, KS) and Dr. Markku Kurkinen (Department of Medicine, UMDNJ-Robert Wood Johnson Medical School). After DNA sequence verification, this DNA fragment was ligated into the expression vector, pET-3d (Novagen, Madison, WI), and expressed in *E. coli* BL21 (DE3) cells as previously described (Marcy, A. I. et al. *Biochemistry* **1991**, *30*, 6476−6483). ProMMP-3 was solubilized form inclusion bodies in 8 M guanidine-HCl, refolded in 100 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$, and 0.5 mM zinc acetate, and activated overnight at 37 °C with 1.5 mM *p*-aminophenylmercuric acetate. Active, truncated MMP-3 was purified to homogeneity over a hydroxamic acid inhibitor affinity column as described (Moore, W. A.; Spilburg, C. A. *Biochemistry* **1986**, *25*, 5189−5195). N-Terminal sequence analysis confirmed the sequence to be consistent with the catalytic domain, Phe^{83}-Thr^{255}, of proMMP-3.

MMP Inhibition Assay. Inhibition of all enzymes was measured according to the representative procedure described below for MMP-2. ProMMP-1 was activated prior to assay by treatment with trypsin. ProMMP-2 was activated prior to assay by treatment with 1 mM *p*-aminophenyl-mercuric acetate for 45 min at 37 °C. ProMMP-3 was activated prior to assay by treatment with *p*-aminophenylmercuric acetate or trypsin. ProMMP-9 was activated with MMP-3 (1:20, MMP-3:MMP-9) and stored at −80 °C until use. MMP-7, 8, and 13 were all supplied as active enzymes and stored frozen until use. MMP activity was monitored using a fluorescence assay previously described,[15] modified for a microtiter plate format. In a Dynatech MicroFLUOR plate, active enzyme was incubated with 8 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 in 50 mM Tris-HCl pH 7.5, 10 mM $CaCl_2$, 0.15 M NaCl, 0.05% Brij for 20−30 min at 37 °C in the presence of varying concentrations of inhibitor. The reaction was then quenched with 50 mM EDTA, and the relative fluorescence was monitored on a Perkin-Elmer LS50B spectrofluorometer (λex 328 nm, λem 393 nm) fitted with a microplate reader attachment. Activity was measured as a percentage of control activity in the absence of inhibitor. Inhibitor concentrations were run in triplicate, and IC_{50} determinations were calculated from a four-parameter logistic fit of the data within a single experiment.

In Vitro Intestinal Permeability Studies.[21] Briefly, a portion of rat ileum was freshly excised and mounted in an in vitro diffusion chamber system (NaviCyte, San Diego, CA). The amount of drug transported across the tissue with time at various donor concentrations of drug was determined at 37 °C and pH 7.4 in modified Krebs bicarbonate buffer. Permeability coefficients, k_p (cm/min) were calculated at steady state with the following equation from Fick's laws of diffusion

$$k_p = [S]/[AC_o]$$

where S is the slope of the linear (steady state) portion of the cumulative amount transported through the membrane versus time plot in μg/min, A is the cross-sectional area of the tissue surface exposed to solute transport in cm^2 (0.636 cm^2), and C_o is the initial concentration of solute in the donor chamber in μg/mL (approximately 25−65 μg/mL for compounds tested). A predicted percent absorption, Abs, is obtained from the ratio of the k_p value for the test compound divided by the k_p of the mannitol internal standard (k_p ratio) from the following equation:

$$\text{Abs} = [100]/[1 + \exp\{-7.42(k_p \text{ ratio} - 0.812)\}]$$

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References

(1) (a) Lafleur, M.; Underwood, J. L.; Rappolee, D. A.; Werb, Z. Basement Membrane and Repair of Injury to Periferal Nerve; Defining a Potential Role for Macrophages, Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases-1. *J. Exp. Med.* **1996**, *184*, 2311−2326. (b) Buisson, A. C.; Gilles, C.; Polette, M.; Zahm, J. M.; Birembout, P.; Tournier, J. M. Wound Repair-Induced Expression of Stromelysins is Associated with the Acquisition of a Mesenchymal Phenotype in Human Respiratory Epithilial Cells. *Lab. Invest.* **1996**, *74*, 658−669. (c) Woessner, J. F., Jr. Metalloproteinases and Their Inhibitors in Connective Tissue Remodeling. *FASEB J.* **1991**, *5*, 2145−2154.

(2) Folkman, J.; Shing, Y. Angiogenesis *J. Biol. Chem.* **1992**, *267*, 10931−10934.

(3) Cawston, T. Metalloproteinase Inhibitors and the Prevention of Connective Tissue Breakdown. *Pharmacol. Ther.* **1996**, *70*, 163−182.

(4) (a) Ahrens, D.; Koch, A. E.; Pope, R. M.; Steinpicarella, M.; Niedbala, M. J. Expression of Matrix Metalloproteinase 9 (96-kd Gelatinase B) in Human Rheumatoid Arthritis. *Arthritis Rheum.* **1996**, *39*, 1576−1587. (b) Blaser, J,; Triebel, S.; Maajosthusmann, U.; Romisch, J.; Krahlmateblowski, U.; Freudenberg, W.; Fricke, R.; Tschesche, H. Determination of Metalloproteinases, Plasminogen-Activators and their Inhibitors in the Synovial Fluids of Patients with Rheumatiod Arthritis During Chemical Synoviorthesis. *Clin. Chim. Acta* **1996**, *244*, 17−33.

(5) Overall, C. M.; Wiebkin, O. W.; Thonard, J. C. Demonstration of tissue Collagenase Activity in vivo an its Relationship to Inflammation Severity in Human Gingiva. *J. Periodontal Res.* **1987**, *22*, 81−88.

(6) (a) Yong, V. W. The potential Use of MMP Inhibitors to Treat CNS Diseases. *Expert Opin. Invest. Drugs* **1999**, *94*, 2177−2182. (b) Maeda, A.; Sobel, R. A. Matrix Metalloproteinases in the Normal Human Nervous System, Microglial Nodules, and Multiple Sclerosis Lesions. *J. Neuropathol. Exp. Neurol.* **1996**, *55*, 300−309. (c) Chandler, S.; Coates, R.; Gearing, A.; Lury, J.; Wells, G.; Bone, E.; Matrix Metalloproteinases Degrade Myelin Basic Protein. *Neurosci. Lett.* **1995**, *201*, 223−226. (d) Hewson, A. K.; Smith, T.; Leonard, J. P.; Cuzner, M. L. Suppression of Experimental Allergic Encephalomyelitis in the Lewis Rat by the Matrix Metalloproteinase Inhibitor Ro31-9790. *Inflamm. Res.* **1995**, *44*, 345−349.

(7) (a) Brown, P. D. Matrix Metalloproteinases in Gastrointestinal Cancer. *Gut* **1998**, *43*, 161−163. (b) Chambers, A. F.; Matrisan, L. M. Changing Views of the Role of Matrix Metalloproteinases in Metastasis. *J. Natl. Cancer Inst.* **1997**, *89*, 1260−1270. (c) Yu, A. E.; Hewitt; R. E.; Connor, E. W.; Stetler-Stevenson, W. G. Matrix Metalloproteinases: Novel Targets for Directed Cancer Therapy. *Drugs Aging* **1997**, *11*, 229−244. (d) Zuker, S.; Lystik, R. M.; Zarrabi, H. M.; Moll, U.; Tickle, S. P.; Stetler-Stevenson, W.; Baker, T. S.; Docherty, A. J. P. Plasma Assay of Matrix Metalloproteinases (MMP's) and MMP−Inhibitor Complexes in Cancer. *Ann. N.Y. Acad. Sci.* **1994**, *732*, 248−262.

(8) (a) Beckett, R. P.; Whittaker, M. Matrix Metalloproteinase Inhibitors 1998. *Expert Opin. Ther. Patents* **1998**, *8*, 259−282. (b) White, A. D. Bocan, T. M. A.; Boxer, P. A.; Peterson, J. T.; Schrier, D. Emerging Therapeutic Advances for the Development of Second Generation Matrix Metalloproteinase Inhibitors. *Curr. Pharm. Des.* **1997**, *3*, 45−58. (c) Zask, A.; Levin, J. I.; Killar, L. M.; Skotnicki, J. S. Inhibition of Matrix Metalloproteinases: Structure Based Design. *Curr. Pharm. Des.* **1996**, *2*, 624−661.

(9) (a) Cherney, R. J.; Wang, L.; Meyer, D. T.; Xue, C.-B.; Arner, E. C.; Copeland, R. A.; Covington, M. B.; Hardman, K. D.; Wasserman, Z. R.; Jaffee, B. D.; Decicco, C. P. Macrocyclic Hydroxamate Inhibitors of Matrix Metalloproteinases and TNF-α Production. *Biorg. Med. Chem. Lett.* **1999**, *9*, 1279−1284. (b) Natchus, M. G.; Cheng, M.; Wahl, C. T.; Pikul, S.; Almstead, N. G.; Bradley, R. S.; Taiwo, Y. O.; Mieling, G. E.; Dunaway, C. M.; Snider, C. E.; McIver, J. M.; Barnett, B. L.; McPhail, S. J.; Anastasio, M. B.; De, B. Design and Synthesis of Conformationally-Constrained MMP Inhibitors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2077.

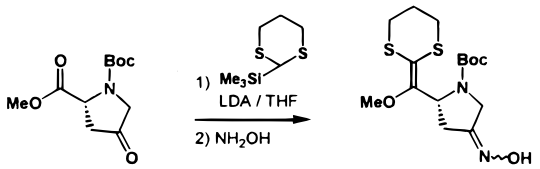
(10) (a) MacPherson, L. J.; Parker, D. T. New Aryl-sulfonyl-aminohydroxamic Acid Derivatived − Useful as Metalloprotease Inhibitors for Treating Arthritis, Ulcers, Periodontal Disease, Tumor Metastasis, HIV Infections, etc. European Patent Application. EP606046A, 1993. (b) MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V. S.; Parker, D. T. Discovery of CGS 27023A, a Non-Peptidic, Potent, and Orally Active Stromelysin Inhibitor That Blocks Cartilage Degradation in Rabbits. *J. Med. Chem.* **1997**, *40*, 2525−2532.

(11) (a) Pikul, S.; McDow-Dunaway, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; McPhail, M. V.; Snider, C. E.; Taiwo, Y. O.; Rydel, T.; Dunaway, C. M.; Gu, F.; Mieling, G. E. Discovery of Potent, Achiral Matrix Metalloproteinase Inhibitors. *J. Med. Chem*. **1998**, *41*, 3568. (b) Almstead, N. A.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. Design, Synthesis and Biological Evaluation of Potent Thiazine and Thiazapine Based MMP Inhibitors. **1999**, in press.

(12) (a) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons, Inc.: New York, 1967; Vol. 1, p 478. (b) Hauser, C. R.; Renfrow, W. B., Jr. *Org. Synth. Coll. Vol.* **1943**, *2*, 67.

(13) (a) Hudlicky, T.; Zingde, G. S.; Natchus, M. G.; Ranu, B. C.; Papadopoulos, P. J. System Oriented Design of Triquinanes: Stereocontrolled Synthesis of Pentallenic Acid and Pentalenene. *Tetrahedron* **1987**, *43*, 5685. (b) Conia, J. M., Limasset, J. C. *J. Bull. Soc. Chim. Fr.* **1967**, *6*, 1936.

(14) In the course of our olefination experimentation we observed a curious electrophilic preference of the dithiane anion below for an ester moiety over a ketone moiety. The structure of the product was confirmed by X-ray crystallography. For reference, see: (a) Muzard, M.; Portella, C. *Synthesis* **1992**, 965. (b) Carey, F. A.; Court, A. S. *J. Org. Chem*. **1972**, *37*, 1926.



(15) Knight, C. G., Willenbrock, F., Murphy, G. A. A Novel Coumarin-labeled Peptide for Sensitive Continuous Assays of the Matrix Metalloproteinases. *FEBS Lett*. **1992**, *296*, 263−266.

(16) Okada, Y.; Nagase, H.; Harris, E. D. A Metalloproteinase from Human Rheumatiod Synovial Fibroblasts that Digests Connective Tissue Matrix Components. Purification and Characterization. *J. Biol. Chem*. **1986**, *261*, 14245−14255.

(17) There is general knowledge that sulfonyl substituents go into the P1′ pocket which is much more shallow for MMP-1 and -7 than the other enzymes of the family. Bode, W.; Fernandez-Catalan, C.; Grams, F.; Gomis-Ruth, F.-Z.; Nagase, H.; Tscheche, H.; Maskos, K. Insights into MMP-TIMP Interactions. *Ann. N.Y. Acad. Sci*. **1999**, *878*, 73−91.

(18) Protein Data Bank file name: 1D7X.

(19) (a) See ref 12. (b) Babine, R. E.; Bender, S. L. Molecular Recognition of Protein−Ligand Complexes: Applications to Drug Design. *Chem. Rev*. **1997**, *97*, 1359−1472. (c) Gonella, N. C.; Li, Y.-C.; Zhang, X.; Paris, C. G. Bioactive Conformation of a Potent Stromelysin Inhibitor Determined by X-nucleus Filtered and Multidimensional NMR Spectroscopy. *Bioorg. Med. Chem*. **1997**, *5*, 2193−2201.

(20) It has been demonstrated in the literature that the *R*-oriented oxygen plays a more significant role in binding than does the *S*-oriented oxygen, and this is believed to be due to its closer relative distance to the enzyme: Pikul, S.; McDow-Dunham, K.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Chen, L.; Dunaway, C. M.; Gu, F.; Mieling, G. E. Design and Synthesis of Phosphinamide-Based Hydroxamic Acids as Inhibitors of Matrix Metalloproteinases. *J. Med. Chem*. **1999**, *42*, 87−94.

(21) Dowty, M. E.; Dietsch, C. R. Improved Prediction of *In-Vivo* Peroral Absorption from *In-Vitro* Intestinal Permeability Using an Internal Standard to Control for Intra- and Inter-Rat Variability. *Pharm. Res*. **1997**, *14*, 1792−1797

(22) Desai, S. R.; Liu, D. Z.; Thakker, D. R. The Unusual Effect of Pyridyl Group on the Transcellular Transport of Compounds Across the Caco-2 Cell Monolayers. *Pharm. Res*. **1997**, *14*, S-21.

(23) Cremlyn, R. J.; Cronje, T.; Goulding, K. Some Phenoxybenzenesulfonyl Chlorides and Related Compounds. *Aust. J. Chem*. **1979**, *32*, 445−452.

(24) Schraufstatter, E.; Schmidt-Kastner, G.; Wirth, W. Ger. Patent 1,078,129.

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